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Invited review

Response of brain tissue to chronically implanted neural electrodes

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Abstract

Chronically implanted recording electrode arrays linked to prosthetics have the potential to make positive impacts on patients suffering from full or partial paralysis. Such arrays are implanted into the patient's cortical tissue and record extracellular potentials from nearby neurons, allowing the information encoded by the neuronal discharges to control external devices. While such systems perform well during acute recordings, they often fail to function reliably in clinically relevant chronic settings. Available evidence suggests that a major failure mode of electrode arrays is the brain tissue reaction against these implants, making the biocompatibility of implanted electrodes a primary concern in device design. This review presents the biological components and time course of the acute and chronic tissue reaction in brain tissue, analyses the brain tissue response of current electrode systems, and comments on the various material science and bioactive strategies undertaken by electrode designers to enhance electrode performance.

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1. Introduction

Reports that monkeys accurately and reproducibly controlled a robotic arm via chronically implanted cortical electrodes rekindled the hope of approximately 200,000 patients suffering from full or partial paralysis in the U.S. (Carmena et al., 2003). The implants, made out of dozens of wire electrodes, sampled extracellular potentials from portions of the monkeys' cortex (Fig. 1). The potentials recorded from neurons adjacent to the electrodes were correlated to observed physical motion, eventually allowing the researchers to translate the neuronal activity directly into robotic arm movements. Because of the length of time required for training and the eventual clinical necessity of a long-term brain–machine interface, the implants were chronic, that is they were permanently implanted into the monkeys' brains.

The purpose of these implanted devices is to record signals with high signal to noise ratios (SNR) from as many individual neurons, termed single units, as possible. Single units must be separated from the background electrical noise and from the overlapping signals, or multi-unit potentials, of other nearby neurons. Principle component analysis and other signal processing techniques can be used to separate single units from noise and multi-unit potentials (Williams et al., 1999b), with a target SNR for recordings around 5:1 (Maynard et al., 2000; Rousche et al., 2001).

A number of engineering groups are developing multi-channel recording electrode arrays for chronic applications. These designs span several different technologies, including microwires (Williams et al., 1999a; Kralik et al., 2001), polymers (Rousche et al., 2001), and different types of silicon micromachined implants (Drake et al., 1988; Campbell et al., 1991). The basic sensor and instrumentation requirements of such neural implant systems are well established, and in general, the present designs perform as intended in short term studies. However, many designs perform inconsistently in chronic applications, a problem that poses a significant barrier to the clinical development of this promising technology.

For example, Nicolelis et al. (2003) reported a 40% drop in the number of functional electrodes between 1 and 18 months. Only 7 of 11 electrode shafts in Rousche and Normann's implanted array recorded signals at implantation, and the number of electrodes recording signals dropped to 4 of 11 after 5 months (Rousche and Normann, 1998). In a study by Williams et al. (1999a), eight cats were implanted with microwire electrode arrays and electrical activity was recorded over time. Three of the electrode arrays failed within the first 15 weeks, presumably due to the tissue reaction and loosening of the skull cap used to keep the electrode in place. The other electrode arrays remained active until the cats succumbed to unrelated medical complications between 15 and 25 weeks post-implantation. Liu et al. (1999) found a large amount of variation in the stability of neural recordings between different electrode shafts on the same array and between arrays implanted in different cats. Most neuronal recordings in the study either grew in stability until day 80 post-implantation, after which the recording remained stable, or degenerated from a high stability to nearly no signals around 60 days post-implantation.

These studies are reminders of the different requirements of a chronic brain implant suitable for use in basic research and the reliability threshold needed for clinically relevant neuroprosthetics. For clinical applications, implanted microelectrode arrays intended as control and communication interfaces need to record unit activity for time periods of the order of decades (Nicolelis and Ribeiro, 2002) and must have a substantially high performance reliability.

This review highlights what is known of the tissue reaction to implantable electrodes, commonly referred to as the “biocompatibility” of the implant. In addition, we survey some of the potential strategies used to improve the biocompatibility of chronically implanted cortical electrodes. The purpose of the review is not to provide an exhaustive examination into any one research thrust, but rather to present a broad view of the issues surrounding this biocompatibility problem so that computational neuroscientists, biomedical engineers, materials scientists, and neurobiologists can come together with a common understanding of the issues and advance the field.

2. Immune response to electrode-like implants in the brain

Available studies suggest that the greatest challenge to obtaining consistent or stable intracortical recordings is the biological response that the brain mounts against implanted electrodes. In order to design recording electrodes that minimize or evade the tissue response of the central nervous system (CNS), it is necessary to understand the biological mechanisms involved. The following provides an overview of the CNS tissue response to implanted needle-like materials. For a more in-depth analysis, the reader is referred to Landis (1994) or Berry et al. (1999).

2.1. Cells involved in the brain tissue response

There are several distinct cell populations involved in the inflammatory and wound healing response to materials implanted in the CNS, also referred to as the “foreign body response.” The cell most commonly associated with brain tissue is the neuron. The location of the neuron soma and its cellular processes relative to the electrode recording sites determines the strength and quality of the recorded electrical signal. Theoretical models predict that action potentials cannot be observed above noise farther than approximately 130 μm from a recording site and that the neuron soma contributes the majority of the recorded signal (Eaton and Henriquez, 2005). Direct measurements suggest however, that the maximum distance is much less, somewhere between 50 and 100 μm (Mountcastle, 1957; Rall, 1962; Rosenthal, 1972; Henze et al., 2000). Thus, the distance required to maintain a recording between an electrode site and a neuron cell body is of the order of cell dimensions.

Although neuronal networks are responsible for information processing and ultimate control of bodily functions, neurons make up less than 25% of the cells in the brain (Purves et al., 2001). The remaining tissue consists of the glial cells (oligodendrocytes, astrocytes, and microglia) and vascular-related tissue.

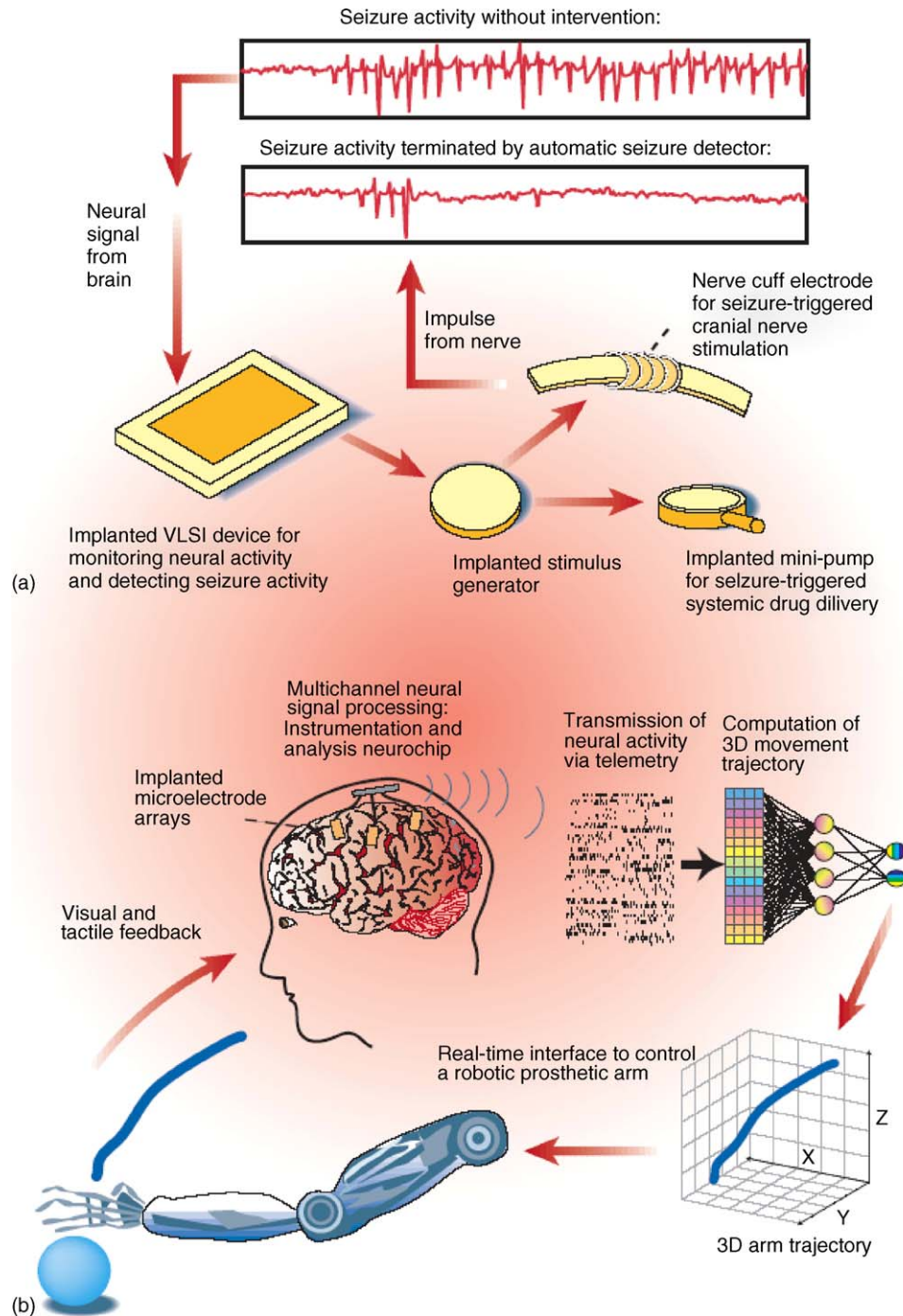


Fig. 1. Schematic description of two potential applications of brain-machine interfaces. (a) A “brain pacemaker” that monitors neural activity to detect seizures. When seizures activity is detected, the implant sends a signal to a nerve cuff electrode or a mini-pump for drug delivery to stop the seizures. (b) Electrode arrays sample the activity of large populations of neurons to control the movements of a prosthetic arm (from Nicoletis, 2001).

Oligodendrocytes are the myelin forming cells of the CNS, while astrocytes and microglia are the main effectors of the brain’s response to injury.

Astrocytes make up 30–65% of the glial cells in the CNS (Nathaniel and Nathaniel, 1981). They contain an elaborate set of cellular extensions, giving them a star-like appearance in histological preparations of brain tissue sections and in cell culture. They provide growth cues to neurons during CNS development,

mechanically support the mature neuronal circuits, help control the chemical environment of the neurons, buffer the neurotransmitters and ions released during neuronal signaling, and even modulate the firing activity of neurons (Kimelberg et al., 1993; Kettenmann and Ransom, 1995; Purves et al., 2001). Specialized astrocytic extensions, termed end feet, abut capillary walls and aid in the transfer of nutrients across the blood–brain barrier. Similar processes weave together to form the 15 μm thick

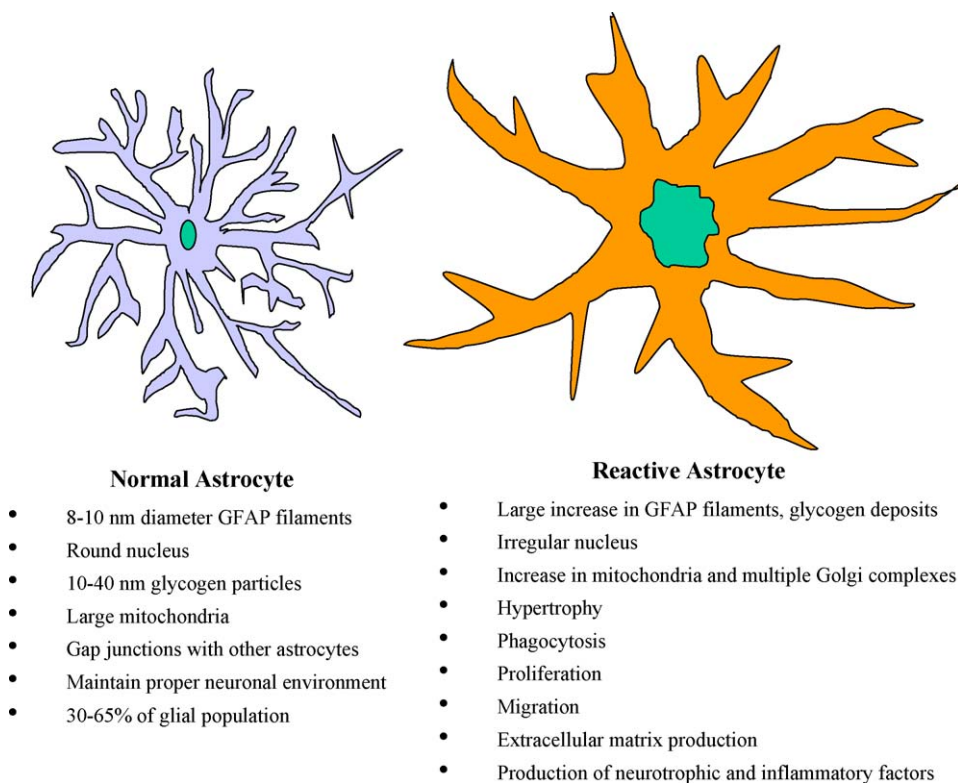


Fig. 2. Schematic representation of astrocyte activation to a reactive phenotype.

glia limitans, the glial boundary between CNS and non-CNS structures that can be clearly seen even between the CNS and the peripheral nervous system (PNS) (Nathaniel and Nathaniel, 1981). Astrocytes are characterized by 8–10 nm diameter intermediate filaments of polymerized glial fibrillary acid protein (GFAP), which is considered an astrocyte specific cell marker

(Eng and DeArmond, 1982). Activation of the astrocytes by injury transforms the cells into a “reactive” phenotype (Fig. 2) characterized by enhanced migration, proliferation, hypertrophy, upregulation of GFAP, changes in the number, and distribution of cellular organelles and glycogen deposits, and increased matrix production (Landis, 1994). Immunostaining for GFAP

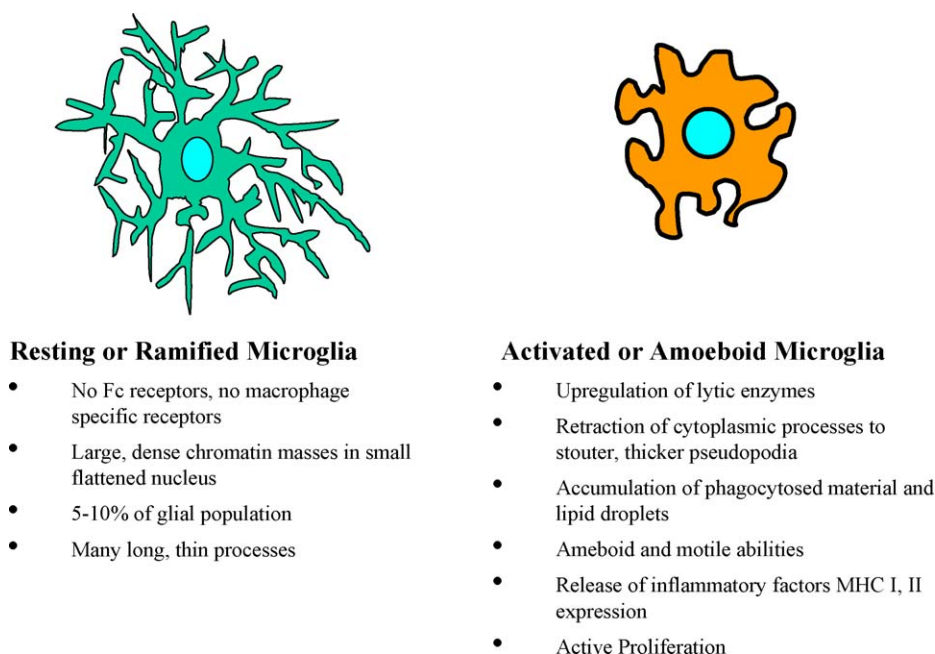


Fig. 3. Schematic representation of microglial transformation from a resting to an activated, mobile, highly phagocytic cell.

is the most common method for astrocyte identification and for determining the extent of “reactive gliosis”, the term used to describe the activation, hypertrophy, and proliferation of astrocytes in response to injury (Bignami et al., 1980; Eng and DeArmond, 1982).

Microglia are the other major glial cell type involved in the brain’s wound healing response, constituting 5–10% of the total number of glial cells in the brain (Ling, 1981). These cells appear to arrive in the brain via a prenatal infiltration of the CNS by blood-borne hematopoietic cells and remain thereafter as the resident macrophages in neural tissue. Primarily, they act as cytotoxic cells killing pathogenic organisms or as phagocytes secreting proteolytic enzymes to degrade cellular debris and damaged matrix after injury or during regular cell turnover (Streit, 1995; Purves et al., 2001). Microglia reside in an inactive or ramified, highly branched state until “activated” via injury mediated mechanisms (Fig. 3). Upon activation, they begin to proliferate, assume a more compact “amoeboid” morphology, phagocytose foreign material, and upregulate the production of lytic enzymes to aid in foreign body degradation (Ling, 1981). When CNS damage severs blood vessels, microglia are indistinguishable from the blood borne, monocyte-derived macrophages that are recruited by the degranulation of platelets and the cellular release of cytokines. Macrophage-like cells arriving from damaged blood vessels, those present as perivascular resident cells, and microglia seem to perform the same functions in response to injury and will not be distinguished hereafter in this review.

Microglia are also known to secrete multiple soluble factors that affect a variety of processes and signaling pathways, which makes it difficult to understand their precise role in the brain tissue response to implanted materials (Banati et al., 1993). They are a potent source of MCP-1, a chemokine that recruits macrophages and activated microglia (Babcock et al., 2003), and of pro-inflammatory cytokines, such as IL-1 (Giulian et al., 1994a), IL-6 (Woodroffe et al., 1991), and TNF- α (Giulian et al., 1994b; Sheng et al., 1995; Chabot et al., 1997). Furthermore, microglia are known to secrete, either constitutively, or in response to pathological stimuli, neurotrophic factors that aid in neuronal survival and growth (Nakajima et al., 2001), including NGF (Elkabes et al., 1996; Nakajima et al., 2001), BDNF (Nakajima et al., 2001), and NT-3 (Elkabes et al., 1996). Finally, microglia produce various cytotoxic and neurotoxic factors (reviewed by Nakajima et al., 2001) which can lead to neuronal death in vitro (Giulian et al., 1994a) and in vivo following traumatic injury (Giulian et al., 1993a,b). These cytotoxic factors include excitatory amino acids, such as glutamate, reactive oxygen intermediates (ROIs) generated as a result of the microglial “respiratory burst” (Auger and Ross, 1992), such as hydrogen peroxide (H_2O_2) or superoxide anion ($\text{O}_2^{\cdot-}$) (Giulian and Baker, 1986), and reactive nitrogen intermediates, such as nitric oxide (NO) (Zielasek et al., 1996; Minghetti and Levi, 1998). It has been suggested that the presence of insoluble materials in the brain may lead to a state of “frustrated phagocytosis,” or an inability of macrophages to remove the foreign body, which results in a persistent, constitutive release of neurotoxic substances (Weldon et al., 1998).

2.2. Assessing biocompatibility

The literature reflects a concerted effort to identify and characterize the changing temporal characteristics of the foreign body response in the CNS. The most common method to assess the tissue response is to implant model probes into test animals and sacrifice the animals at various time points to evaluate the probe and adjacent neural tissue. Direct evidence linking tissue response to device performance, however, has not been studied extensively. The majority of available evidence is derived from studies that examine the brain tissue response to passive or non-functional implants. Because biocompatibility is a functional characteristic, which strictly speaking requires an analysis of tissue response using an electrically functional implant, the effect of the tissue response on device function is mostly inferred.

In general, electrodes are removed following tissue fixation and the excised tissue surrounding the implant is sectioned and stained to identify cell numbers, locations, types, and by-products. For the most part, the results are subjective, consisting of a description of a particular stain in comparison to adjacent, uninjured tissue from a few selected sections with the number of animals per time point and number of sections analysed varying considerably from study to study. A few studies have examined the explanted electrodes with histological methods (Turner et al., 1999; Szarowski et al., 2003; Biran et al., 2005). Most of the histological studies have focused on the reactive astrogliosis, and less so on the microglial response or on the effect of the response on the surrounding neuronal population.

Early studies relied on Hematoxylin and Eosin (H&E) to determine the location of neurons and other relevant cell types relative to the implant (Edell et al., 1992), although H&E is not a cell-type-specific stain. To gain more specificity, immunostaining for cell-type-specific proteins, such as GFAP for astrocytes, and NeuN or MAP-2 for neurons, is used to localize cells and to determine their state of activation (Menei et al., 1994). Following activation, microglial cells exhibit increased expression of certain leukocyte-associated molecules, including CD68 (recognized by the ED-1 antibody) and Mac-1/CD11b (recognized by the OX-42 antibody), or of various lectins (Thomas, 1992). A stain for vimentin, an intermediate filament expressed in reactive astrocytes, microglia, and perivascular cells, can also be employed (Cui et al., 2003) although specificity is lost. To test the brain’s response to acute injury, as opposed to chronic implantation, the implant can be removed soon after implantation, or merely used to create the initial insertion injury without actual implantation (Yuen and Agnew, 1995; Biran et al., 2005).

Although staining allows for the identification of reactive cells around an implant, other methods have been used to examine the extracellular environment. Microdialysis sampling allows the user to assess the chemical environment surrounding the implant without the presence of confounding cells (Khan and Michael, 2003). The dialysis membrane can be adjusted to pass only certain molecular weight compounds, and the collected dialysate is then tested (often through an immunoassay) to determine the presence of different compounds in question. In a modification of the microdialysis sampling paradigm, hollow fiber membranes (HFM) of poly(acrylonitrile-vinyl chloride)

(PAN-PVC) have been implanted to mimic electrodes (Kim et al., 2004). The foreign body response around an HFM implant is then characterized by immunostaining for cells and testing extracellular milieu collected from the hollow inner compartment. Finally, others have studied the neural tissue response to implants using hippocampal slice cultures grown on porous membranes or on silicon chips with recording capabilities with variable success (Kunkler and Kraig, 1997; Kristensen et al., 2001).

2.3. Mechanical trauma of insertion

Few studies have examined the initial impact of insertion, and it appears that the first encounter of cortical tissue with a needle-like electrode is a violent one. As the electrode is inserted into the cortex, its path severs capillaries, extracellular matrix, glial and neuronal cell processes. The electrode may pull and snap extracellular matrix materials as it progresses deeper into tissue, push aside tissue that had once occupied the electrode space, and induce a high-pressure region surrounding the electrode.

This mechanical trauma initiates the CNS wound healing response, a response that shares similarities to wound healing responses of other tissues. Disruption of blood vessels releases erythrocytes, activates platelets, clotting factors, and the complement cascade to aid in macrophage recruitment and initiate tissue rebuilding. Insertion induced accumulation of fluid and necrotic nervous tissue causes edema, further adding to the pressure surrounding the implant. When a 10×10 array of silicon probes was implanted in feline cortex, 60% of the needle tracks showed evidence of hemorrhage and 25% showed edema upon explantation of the probes after 1 day (Schmidt et al., 1993). Although a large number of the tracks were affected, only 3–5% of the area was actually covered by hemorrhages and edema, suggesting the actual magnitude of the damage to blood vessels may have been relatively minor. Alternatively, this may have been underestimated by the analytical methods employed.

Activated, proliferating microglia appear around the implant site as early as 1-day post-implantation (Giordana et al., 1994; Fujita et al., 1998; Szarowski et al., 2003). Edema and erythrocytes remain after 4 days post-implantation, although excess fluid and cellular debris diminishes after 6–8 days due to the action of activated microglia and re-absorption (Stensaas and Stensaas, 1976). The presence of erythrocyte breakdown products (but not hemorrhages) and necrotic tissue can still be seen after 6 weeks time (Stensaas and Stensaas, 1976; Turner et al., 1999). At later time points, some report that typical inflammatory cells or hemorrhaging cannot be seen (Yuen and Agnew, 1995), while others have reported observing macrophages at the device brain tissue interface at up to 16 weeks (Stensaas and Stensaas, 1976; Turner et al., 1999; Szarowski et al., 2003; Biran et al., 2005). As testament to the transitory nature of this mechanically induced wound healing response, electrode tracks could not be found in animals after several months when the electrode was inserted and quickly removed (Yuen and Agnew, 1995; Rousche et al., 2001; Csicsvari et al., 2003; Biran et al., 2005), indicating that the persistent presence of the implant augments the brain tissue response.

2.4. Long-term inflammation

The brain tissue response of chronically implanted electrodes would be less of an issue if the foreign body response disappeared a few weeks after implantation as observed with stab wounds. However, once the acute inflammatory response declines, a chronic foreign body reaction is observed. This reaction is characterized by the presence of both reactive astrocytes, which form a glial scar (detailed in the next section), and activated microglia (Stensaas and Stensaas, 1976; Turner et al., 1999; Szarowski et al., 2003; Biran et al., 2005).

In many studies, a significant portion of cells in damaged neural tissue do not stain for GFAP, suggesting the presence of large numbers of activated microglia at the surface of implanted biomaterials long after the initial wound healing response is complete, and perhaps as long as the material remains in contact with brain tissue (Stensaas and Stensaas, 1976, 1978; Winn et al., 1989; Edell et al., 1992; Menei et al., 1994; Kunkler and Kraig, 1997; Mofid et al., 1997; Emerich et al., 1999; Mokry et al., 2000; Szarowski et al., 2003). For example, nearly 25% of electrode tracks in a multi-pronged silicon probe array showed macrophage-like cells present 6 months after implantation (Schmidt et al., 1993). Activated microglia will attempt to phagocytose foreign matter for eventual degradation. When 25 μ m polymeric microspheres were implanted into rat cortex, they were all phagocytosed by the activated microglia within 2 months and remained internalized throughout the remainder of the 9-month study (Menei et al., 1994). Immunostaining around a needle-like implant revealed scattered reactive microglia in the initial wound healing response, clustering of microglia in a reactive tissue sheath forming around the implant after 2 weeks, and continued presence of microglia in a tight cellular sheath at 12 weeks post-implantation (Szarowski et al., 2003). Cells not staining for GFAP had adhered to the implant upon its extraction in the same study.

When macrophages outside the CNS encounter a foreign object, they surround it and begin secreting lytic enzymes. If individual macrophages cannot degrade the object, these cells often fuse into multi-nucleated foreign body “giant” cells characteristic of chronic inflammation. This closely parallels the activated microglial reaction to electrode implants in the CNS. Needles made out of a plastic used for tissue mounting (Araldite) implanted into rabbit cortex attracted variably sized multi-nucleated “giant” cells as early as 18 days post-implantation (Stensaas and Stensaas, 1976). These cells were separated from other cortical tissue by a basal lamina and were mostly found adjacent to degraded regions of the plastic needles, suggesting the action of hydrolase activity. A similar layer of tightly coupled multi-nucleated giant cells was observed by Edell et al. (1992) with cortically implanted silicon electrodes.

A recent study observed persistent ED-1 immunoreactivity around silicon microelectrode arrays implanted in rat cortex at 2 and 4 weeks following implantation that was not observed in microelectrode stab wound controls, indicating that the phenotype was not the result of the initial mechanical trauma induced by probe insertion but was associated with the foreign body response (Biran et al., 2005). In addition, electrodes explanted

at 1, 2, and 4 weeks after implantation were covered with ED-1/OX-42 immunoreactive cells that released MCP-1 and TNF- α in vitro, indicating that inflammation mediated neurotoxic mechanisms may be occurring at the microelectrode brain tissue interface.

2.5. Glial scar formation

The most common observation of the long-term CNS response to chronically implanted electrodes is the formation of an encapsulation layer referred to as the “glial scar” (Edell et al., 1992; Turner et al., 1999; Maynard et al., 2000; Shain et al., 2003; Biran et al., 2005). Studies have demonstrated that reactive glial tissue surrounds and progressively isolates implanted arrays in a process similar to the fibrotic encapsulation reaction that is observed with non-degradable implants in soft tissues of the body. The development of this encapsulation tissue is limited to higher vertebrates and has been implicated in the resistance of the spinal cord and the brain to nerve regeneration after injury (Reier et al., 1983). The purpose of the glial scar remains unclear, but it is thought to play a role in separating damaged neural tissue from the rest of the body to maintain the blood–brain barrier and to prevent lymphocyte infiltration (Nathaniel and Nathaniel, 1981; Landis, 1994). While soft tissue encapsulation involves a variety of cells and their secreted matrix, reactive astrocytes are the major component of CNS encapsulation tissue (Schmidt et al., 1976, 1997; Schultz and Willey, 1976; Agnew et al., 1986; Edell et al., 1992; Carter and Houk, 1993; McCreery et al., 1997; Turner et al., 1999; Szarowski et al., 2003). Current theories hold that glial encapsulation, i.e. gliosis, insulates the electrode from nearby neurons, thereby hindering diffusion and increasing impedance (Schultz and Willey, 1976; Liu et al., 1999; Roitbak and Sykova, 1999; Turner et al., 1999), extends the distance between the electrode and its nearest target neurons (Liu et al., 1999), or creates an inhibitory environment for neurite extension, thus repelling regenerating neural processes away from the recording sites (Stichel and Muller, 1998b; Fawcett and Asher, 1999; Bovolenta and Feraud-Espinosa, 2000).

Turner et al. (1999) used confocal microscopy to show the time course of astrogliosis. Passive silicon electrodes were implanted in the rat cerebral cortex and explanted at 2, 4, 6, and 12-week time points (Fig. 4). At 2 weeks, GFAP staining revealed a reactive astrocyte region surrounding the implants that extended out 500–600 μ m. This region decreased over time, but the layer of cells immediately adjacent to the implant became denser and more organized suggesting contraction around the implant. At 2 and 4 weeks, activated astrocytes around the implant had extended their processes toward the insertion site. The mesh of astrocytic processes became stronger and more compact at 6 and 12 weeks, as suggested by the fact that removal of the implant did not result in the collapse of cellular processes into the implantation tract. Both visual and mechanical inspection of the glial sheath suggested that its formation was complete as early as 6 weeks post-implantation and remained intact as long as the implant remained in situ.

A later study by the same group confirmed this time course (Szarowski et al., 2003). This study found a region of diffuse glial activation as imaged by GFAP staining 100–200 μ m away from the implant site after day 1, and a steady increase of astrocyte activation to 500 μ m away from the implant through 1, 2, and 4 weeks. A more compact sheath formed by 6 weeks and remained constant at 12 weeks, with the actual sheath extending only 50–100 μ m around the insertion site. The investigators also stained for vimentin, which is expressed in reactive astrocytes but not mature astrocytes. Vimentin expression followed a time course similar to GFAP, but revealed fewer positive cells, a spatial distribution closer to the implant (25–50 μ m thick layer), and a completed sheath at 4 weeks post-implantation. It is important to note that in each of the aforementioned studies the electrodes were not functional, that is, they were not connected to an external electrical connector externalized through and attached to the skull. Such untethered electrodes may underestimate the actual reactivity caused by electrically active implants that may transmit forces to the implanted electrode. To date, only studies employing non-specific H&E staining have not observed glial scar formation (Stensaas and Stensaas, 1976; Yuen and Agnew,

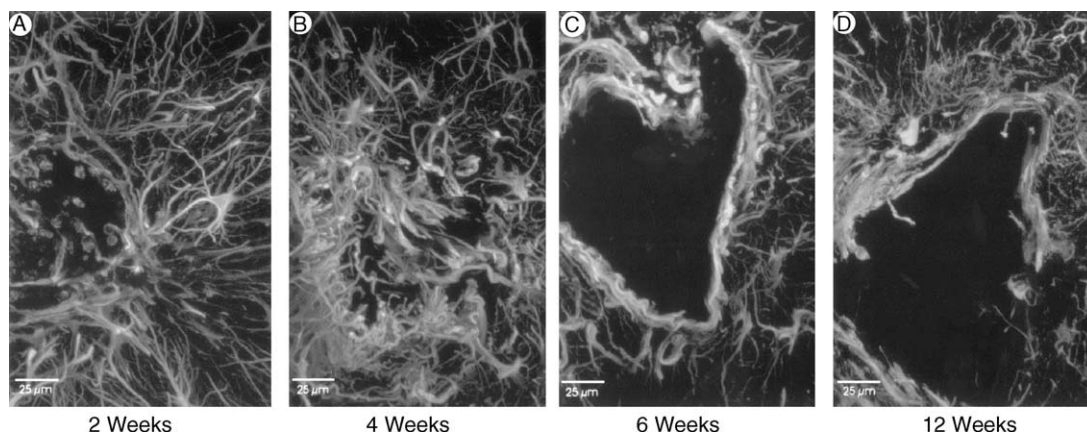


Fig. 4. Time course of glial scar formation at four time points as imaged by GFAP staining. At 2 and 4-week time points, the astrocytic processes fall back into the void left by the probe extraction before tissue processing. By 6 weeks, the processes have interwoven to form a stronger, more dense sheath surrounding the implant. Minimal changes between the 6- and 12-week time points indicate the glial scar completion within 6 weeks (from Turner et al., 1999).

1995; Liu et al., 1999), and in a report where both H&E and GFAP were used on the same samples, it was noticed that H&E staining revealed little gliosis, but GFAP staining clearly showed the development of a brightly stained astrocytic scar around the implant (Maynard et al., 2000).

Other factors and cell types may also contribute to the formation of the glial scar. Several investigators have reported the presence of connective tissue inside this scar similar to the extracellular matrix (ECM) encapsulation seen in wound healing models outside of the CNS (Stensaas and Stensaas, 1976; Liu et al., 1999). Meningeal fibroblasts, which also stain for vimentin, but not for GFAP, may migrate down the electrode shaft from the brain surface and form the early basis for the glial scar (Cui et al., 2003). Support for this fibroblastic role in glial scar formation comes from experiments by Kim et al. (2004) who compared the cellular response to implants completely surrounded by cortical tissue to transcranial implants that also contacted the skull and the meninges (a more accurate model of functional recording electrode arrays). They found a significant increase in ECM and connective tissue in the transcranial probes, as well as a thin layer of GFAP negative/vimentin positive cells surrounding the transcranial probes that was not present in the implants completely surrounded by brain tissue. Staining for ED-1, a microglial marker, confirmed that microglia were present within this one to two cell thick layer, and the authors also concluded that the presence of ECM suggested meningeal fibroblasts had migrated down the probe from the top of the cortex.

2.6. Neuronal response to implant-induced injury

The density of neurons and their proximity to the electrode sites are the most accurate barometers of electrode performance in a chronic setting. Unfortunately, this response is not as well characterized as glial scar formation, as it seems to vary from implant to implant, and even between electrodes implanted at different sites in the same animal. One explanation of electrode signal degradation is the formation of a “kill zone” around the implant site resulting from the initial trauma or neuroinflammatory events (Edell et al., 1992; Biran et al., 2005). This region is defined by a significantly lower or non-existent neuronal density up to some distance away from the electrode. One group found a kill zone of less than 10 μm for electrode shafts that had not induced major trauma, with a larger kill zone of 20–60 μm for shafts that experienced minor lateral motion tangential to the brain surface during implantation (Edell et al., 1992). The authors suggested a correlation between initial tissue damage and kill zone size, although this correlation has not been confirmed by others. Reported kill zone sizes have varied between 1 μm (no dead zone) and more than 100 μm (Stensaas and Stensaas, 1976; Reier et al., 1983; Turner et al., 1999).

An alternative explanation for a loss in signal strength is a slow regression of neurons away from the electrode. Proliferation of astrocytes and formation of the glial scar around the electrodes could be one mechanism by which gliosis displaces neurons near the implant site and pushes cell bodies away from electrode sites (Edell et al., 1992). Liu et al. (1999) implanted

iridium wire electrodes into feline cerebral cortex and tracked the stability of single units over time. Histological examination upon explantation revealed that every electrode with stable unit recordings had at least one large neuron near the electrode tip, while every electrode that was not able to record resolvable action potentials was explanted from a site with no large neurons nearby. The signal strength declined gradually over time for failing electrodes, suggesting a gradual remodeling of the environment rather than neuronal death was responsible for electrode failure. The study also observed that significant changes in recording capability occurred in the first 4–8 weeks post-implantation, after which the signals stabilized (such stabilization also reported in Nicolelis et al., 2003). The authors of the study speculated that electrode migration through tissue resulted in these changes since active restructuring of the adult mammalian brain is severely limited.

A recent study observed a significant loss of neurons around chronically implanted silicon arrays that was not seen in stab wound controls, indicating that the cell loss was associated with the foreign body response (Biran et al., 2005). Immunostaining revealed significant reductions of neurofilament and NeuN at the electrode brain tissue interface that surrounded the implanted electrodes at 2 and 4 weeks following implantation (Fig. 5). In this study although the electrodes were non-functional they were tethered to the skull in a manner similar to working electrodes. In addition, the investigators observed an inverse relationship between persistent ED-1 staining at the microelectrode brain tissue interface and loss of neuronal markers, leading them to speculate that persistent activation of microglia at the device surface leads to local neurotoxicity.

2.7. In vivo experimental variability

The available evidence suggests there is significant variability in electrode performance within experimental groups, between different animal models, investigators, and even between different electrodes implanted in the same animal. There are no common handling, packaging, sterilization, implantation, fixation, or analytical schemes employed. In addition, many of the experiments are improperly controlled, completely subjective and employ too few animals. While such variability is not uncommon for in vivo work of this kind, the different and often conflicting results obtained from these experiments leads to difficulty in drawing meaningful conclusions. For example, in the Edell et al. (1992) study on implanted electrode arrays, electrode shanks with seemingly identical characteristics and insertion techniques resulted in significantly different kill zones. Another confounding example comes from Rousche and Normann (1998) (Fig. 6), who show an H&E stained image of two adjacent electrode tracks from the same electrode array; one track has healthy neurons growing right up against it and no sign of an immune response, while the other very clearly shows the formation of a glial scar and a chronic inflammatory response. Perhaps the clearest example of this variability was observed in the in vivo response to plastic “mock electrodes” implanted in rabbit brain by Stensaas and Stensaas (1976) and explanted over the course of 2 years. They separated the response into three

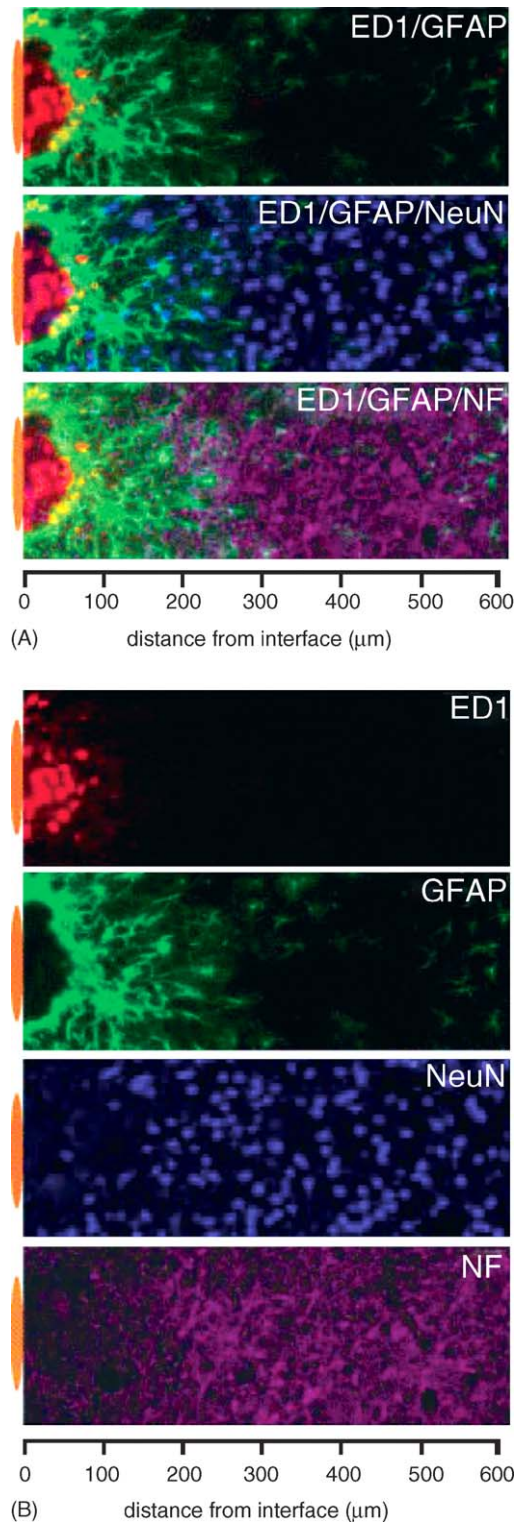


Fig. 5. Stratification of cellular immunoreactivity using cell-type-specific markers at the microelectrode–brain tissue interface. Representative images collected from two adjacent sections of an animal with a 4-week microelectrode implant illustrate the general appearance of the foreign body response characterized by minimally overlapping inflammatory (ED-1) and astrocytic (GFAP) phenotypes adjacent to the implant interface. The area of inflammation and intense astrocyte reactivity contains a reduced number of NeuN⁺ neuronal bodies and a loss of neurofilament (NF) density. The position of the microelectrode is illustrated by the orange oval (drawn to scale) at the left of each image. Images were captured in grayscale and pseudocolored for illustration (from Biran et al., 2005).

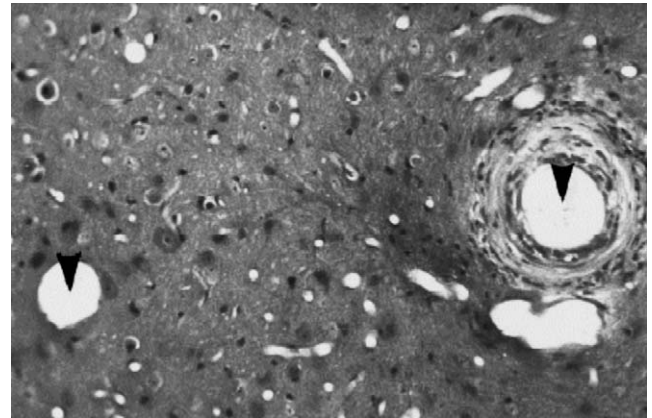


Fig. 6. An example of in vivo variability commonly encountered in implant literature. Arrows show adjacent tracks from two electrodes on the same array. The track on the right clearly shows heavy matrix deposition while that on the left seems to have no tissue reaction (from Rousche and Normann, 1998).

types: Type 1 was characterized by little to no gliosis with neurons adjacent to the implant, Type 2 had a reactive astrocyte zone, and Type 3 exhibited a layer of connective tissue between the reactive astrocyte layer and the implant, with neurons pushed more than 100 μm away. All three responses are well documented in the literature; however this study found that the model electrodes produced all three types of reactions simultaneously, depending on where along the electrode one looked. Although these studies clearly suffer from the insensitivity of the chosen histopathological approach (non-specific tissue staining), it is clear that only a broader view of the literature can yield meaningful conclusions in the face of such experimental variance.

3. Current electrode implant systems

The problems inherent in chronic recording electrode design have precluded the development of a “gold standard” electrode against which testing is performed. Historically, neurobiology research has used single wire or glass micropipette electrodes to record individual neuron waveforms in acute experiments. However, the need to access populations of neurons and the desire of researchers to monitor neuron networks over time has added a new focus on arrays of wires, silicon shafts and other more complex micromachined silicon recording systems capable of high density sampling. Chronic implantation has also generated various surgical techniques aimed at reducing electrode failure and the foreign body response. This section details the most salient features of electrode array design and surgical techniques as they relate to biocompatibility.

3.1. Multiple electrode types

Of the two main types of electrode arrays currently being explored, microwire electrodes have the longest history and widest use in the field. Microwire electrodes are wires made of a conducting metal, such as platinum, gold (Yuen and Agnew, 1995), tungsten (Williams et al., 1999b), iridium (Liu et al., 1999), or stainless steel (Nicolelis et al., 2003), that are coated

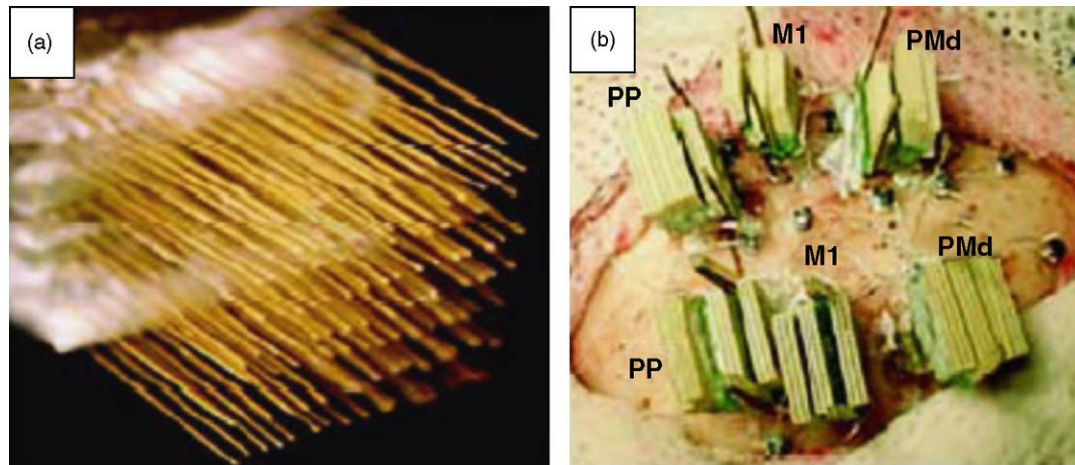


Fig. 7. (a) Wire electrode arrays implanted in macaque monkey cortex. (b) Layout of six such wire arrays in macaque monkey cortex (from Nicolelis et al., 2003).

with a non-cytotoxic insulator material. The tip of the wire is not insulated and can receive electronic signals from the surrounding neurons. In an effort to better separate single unit from multi-unit activity, experimenters often record from two (stereotrode configuration; McNaughton et al., 1983) or four (tetraode configuration; Gray et al., 1995) closely spaced microwires to allow relative signal strength to act as another parameter in single unit identification. Finally, microwires can be arranged in arrays to access the large numbers of neurons necessary for neuroprosthesis control. The number of wires used in a single implanted array has ranged from 4 (Yuen and Agnew, 1995) to over 100 (Nicolelis et al., 2003). A clear advantage of using microwire electrodes is the ease in array fabrication compared to more sophisticated silicon arrays, which are discussed later. Although microwire arrays are simpler, their performance in recording high numbers of single units often exceeds the quality of recordings obtained from silicon-based electrodes. Nicolelis et al. (2003) chronically implanted 10 microwire arrays into macaque monkey cortex for a total of 704 microwires, and were able to record 247 individual cortical neurons in a single session from 384 of the microwires (Fig. 7). While the number of units varied from day to day and between monkeys, the yield of units per recording site was much greater than that of the typical silicon array. Furthermore, microwire arrays can access deeper brain structures, but the precise location of the electrode tips and the interelectrode spacing cannot be controlled as the non-homogeneous nature of brain tissue will bend microwires during implantation (Edell et al., 1992).

Although most neuroscience research continues to be conducted using these well-established microwire electrodes, the next generation of electrode arrays being developed is predominantly silicon based. Silicon micromachined electrodes allow for a more complex design and thus greater flexibility in strategies to minimize the foreign body response and greater control over electrode placement. The emergence of silicon micro-machining technology has yielded increasingly smaller and higher electrode count arrays capable of recording from greater volumes of neural tissue with improved spatial discrimination (Drake et al., 1988; Branner et al., 2001; Csicsvari et al., 2003; Kipke

et al., 2003). However, despite substantial technological advances in their design, many such devices are unreliable for chronic recording applications in the CNS (Liu et al., 1999).

Silicon photolithographic processing allows for unsurpassed control over electrode size, shape, texture, and spacing, allowing multiple recording sites to be placed at variable heights on a single electrode shank. Such control provides the experimenter with absolute knowledge of the recording location, the ability to place the recording sites at different depths to suit the geometry of the neural system under study, and a larger overall number of recording sites on a smaller volume than is possible on wire arrays or bundles (Kewley et al., 1997). Circuits can be integrated directly on the probe for better signal acquisition, and on-chip microelectromechanical systems (MEMS) add additional possibilities, such as heating elements and microfluidics (Chen et al., 1997; Bai and Wise, 2001). Further decreases in electrode sizes and increases in recording site densities are currently limited by connectors and on-board systems that are unable to handle the hundreds of possible leads on a single array (Campbell et al., 1991; Maynard et al., 2000; Bai and Wise, 2001; Kipke et al., 2003).

The designs of silicon-based electrode arrays vary between investigators and research manufacturing centers (Fig. 8) (Edell et al., 1992; Turner et al., 1999; Bai and Wise, 2001; Csicsvari et al., 2003; Szarowski et al., 2003). Nevertheless, two particular silicon electrode array designs have attained prominence in the field. The Utah Electrode Array (UEA) developed by Normann and co-workers has been in use for over 15 years (Campbell et al., 1991; Rousche and Normann, 1998; Maynard et al., 2000). The UEA is created from a single block of silicon which, through etching, doping, and heat treatment, results in a three-dimensional array of needle-like electrodes with recording tips. It has been made in 25 and 100 shank versions of various shapes, with each shank 1.5 mm in length and ranging from 100 μm at its base to less than 1 μm at the tip (as compared to 25–50 μm diameter and up to 8 mm in length for microwires; Nicolelis et al., 2003).

The other prominent electrode design comes from the University of Michigan Center for Neural Communication Technology

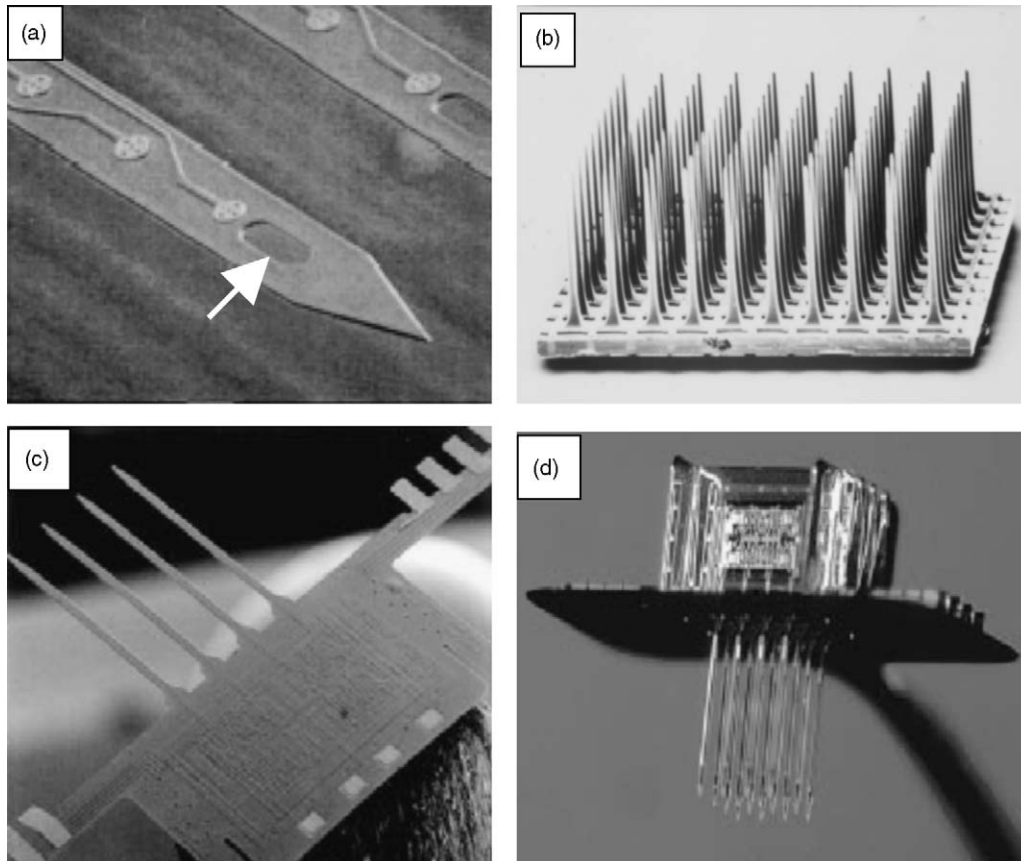


Fig. 8. Various designs of silicon micromachined electrode arrays. (a) Arrow points to a “well” included in the electrode design for bioactive molecule incorporation. Multiple electrode sites are present on each electrode shank (from Kipke et al., 2003). (b) Utah Electrode Array formed from a single block of silicon (from Rousche and Normann, 1998). (c and d) Multiple planar arrays of “Michigan” electrodes are stacked together to create a three-dimensional array (from Bai and Wise, 2001).

(Cui et al., 2001, 2003; Kipke et al., 2003; Szarowski et al., 2003). Unlike the UAE, the Michigan probes are planar arrays of electrode shanks made from a single thin sheet of silicon, but each 2 mm long, $100\ \mu\text{m} \times 15\ \mu\text{m}$ shank has several recording sites placed at user-defined locations along the shank. The manufacturing process is similar to that used to create multi-layer silicon microchips and requires eight masks, but results in hundreds of user-defined, reliable, and regular electrodes from a single block of silicon. A different group has developed a similar design with four recording sites per shank using ceramic rather than silicon for their electrodes, resulting in mechanically stronger electrodes with comparable recording capabilities (Moxon et al., 2004a,b).

3.2. Materials used for the insulating layer

Both microwire and silicon-based electrode array systems require an insulation layer to shield the electrodes from unwanted electrical signals. The vast majority of tissue–electrode contact is with the insulating layer, so this material must be non-toxic and should act to reduce the foreign body response. Several different materials identified as minimally toxic have been used to coat electrodes. A simple coating of Teflon or S-isonel, a high temperature polyester enamel similar to Teflon, has been used with great success to coat wire

electrodes (Kennedy, 1989; Nicolelis et al., 2003). Resins, such as Epoxylite, have also been used successfully (Liu et al., 1999). Plasma-deposited diamond-like carbon (DLC) has recently been demonstrated in vitro as both a chemically inert insulator and as a good substrate for biological molecule attachment to control the foreign body response, although it has not been tested in vivo (Ignatius et al., 1998; Singh et al., 2003).

In addition to the normal silicon nitride or silicon dioxide insulation deposited during the fabrication of micromachined silicon electrodes (Campbell et al., 1991; Kipke et al., 2003), polyesterimide, or more commonly, polyimide is often used to coat silicon-based implants (Campbell et al., 1991; Yuen and Agnew, 1995; Williams et al., 1999b; Bai and Wise, 2001). Lee et al. (2004) reported that fibroblasts spread, adhered, and grew on polyimide electrode surfaces with no difference from tissue culture polystyrene controls. The electrodes were also mounted on a thin ($5\text{--}10\ \mu\text{m}$) silicon substrate to aid in electrode insertion through the pial membrane of the brain (Lee et al., 2004). The flexibility of polyimide may improve the mechanical impedance mismatch between a rigid electrode and soft tissue resulting in tissue damage if micromotion of the electrode occurs (Rousche et al., 2001; Kim et al., 2004). Rousche et al. (2001) eliminated silicon completely and created a flexible electrode out of polyimide and gold, where the gold recording sites and leads were sandwiched between two layers of polyimide. A major drawback

to this design was that the electrodes were not stiff enough to pierce brain tissue on their own, so implant sites had to be created with wire or a scalpel before insertion.

3.3. Electrode insertion and implantation procedure

Many studies have attributed biologically induced electrode failure to the initial trauma of implantation, leading to a variety of strategies to minimize this early trauma in the hope of limiting the subsequent complications. Unfortunately, since each group of investigators works with a different electrode system, a different animal model, and a different set of hands, there is very little consensus regarding the optimal way to implant chronic recording electrodes and a shortage of well-controlled quantitative studies. Approaches differ on the speed of electrode insertion, the method of insertion, the importance of limiting micromotion, and the depth of insertion.

Experimenters use a wide range of insertion speeds for electrode implantation. One theory holds that slow insertion allows for neuronal tissue to adjust to the implant, thus minimizing the damage caused by the electrode. In the experiments conducted by Nicolelis et al. (2003), a 100 $\mu\text{m/s}$ microwire electrode insertion rate was cited as a major factor behind the unusually large number of single units recorded in the study. However, other groups have reported problems with slow insertion, such as catching of the tissue and dural dimpling (Edell et al., 1992). The other school of thought is that a rapid insertion minimizes trauma, since the force of the insertion cuts through the tissue in the array's path, but does not affect nearby tissue (Campbell et al., 1991). Groups using the UEA have found that a high velocity approach (8.3 m/s) prevents cortical surface dimpling and minimizes tissue damage (Campbell et al., 1991; Schmidt et al., 1993; Maynard et al., 2000). Other groups use insertion speeds in between (i.e. 2 mm/s) these two extremes (Turner et al., 1999; Szarowski et al., 2003).

The method of insertion has also been a source of differing opinions. Some groups insert the electrodes by hand (Stensaas and Stensaas, 1976; Yuen and Agnew, 1995; Liu et al., 1999; Williams et al., 1999b; Szarowski et al., 2003), while others utilize microdrives to make the delicate insertions and cite such custom built or commercially available devices as a major factor behind the success of their experiments (Maynard et al., 2000; Csicsvari et al., 2003; Nicolelis et al., 2003; Szarowski et al., 2003). Edell et al. (1992) suggested that maintaining an alignment between the electrode shaft and the axis of insertion was essential by calculating that a 1° misalignment in a 1 mm insertion can cause a 17 μm slash through the tissue at the insertion site, however, no experimental evidence was offered to substantiate this point. Kewley et al. (1997) point to a misalignment of the probe shaft relative to the insertion axis as the major factor contributing to poor signal strengths of some implanted probes in their study.

Several strategies have been employed to minimize the foreign body response via adjustments to electrode implant attachments. One source of electrode micromotion can be the link between the electrode and its connector. A flexible polyimide connection between the rigid electrode and the connector cable was utilized in one set of experiments (Lee et al., 2004), while

another group used a flexible printed circuit board to overcome the perceived problem of micromotion (Kipke et al., 2003). Another set of experiments revealed that adhesions had formed between a UEA implant and the dural tissue above the brain, possibly due to a robust foreign body response mediated by micromotion of the implant relative to the dura (Rousche and Normann, 1998). Placing a Teflon sheet between the array and the dura, and a sheet of Gore-Tex[®] between the dura and the cranium in subsequent experiments significantly improved the performance of the electrode array over the course of 9 months (Maynard et al., 2000). The Teflon sheet also affected implant migration within cortical tissue, a cause of signal degradation cited by another group that had observed longer microwires losing neuronal signals before their shorter counterparts within the same electrode array (Liu et al., 1999).

4. Strategies to minimize the immune response to implanted electrodes

With different electrode array technologies, machining options, biocompatible materials, and implantation procedures available, various groups have altered the design of electrodes in an attempt to minimize or evade the immune response. Investigators better acquainted with the molecular biology of the neural environment have also added bioactive agents to the material science repertoire of electrode designers. This intersection of neural immunobiology and electrode design holds considerable promise for developing reliable and useful probes.

4.1. Material science strategies

The materials science and biocompatibility of packaging materials for sensors outside of the CNS has been reviewed (Sharkawy et al., 1997, 1998a,b; Wisniewski et al., 2000). The majority of previously attempted strategies for limiting the immune reaction to electrodes implanted within the CNS also revolve around material science and physical/mechanical approaches. Electrode size, shape and cross-sectional area have been modified to elicit the smallest possible tissue response. Some reports give significant weight to the texture of an implant (Rousche et al., 2001). Other studies stress the importance of electrode tip shape (Edell et al., 1992; Nicolelis et al., 2003). However, a recent study by Szarowski et al. (2003) downplayed the importance of electrode shape, size, texture, and tip geometry. The study compared the immune response to silicon implants of different sizes, surface characteristics, and insertion techniques (Fig. 9) through GFAP, vimentin, and ED-1 immunostaining over the course of 12 weeks. Electrodes of three sizes (2500, 10,000, and 16,900 μm^2 cross-sections), three cross-sectional shapes (trapezoid, square, and ellipse), two surface textures (smooth and rough), two tip geometries (sharp blade-like point and rounded tip), and two insertion methods (hand and precision drive) were tested in rats. Glial staining revealed that while there were minor temporal differences (of the order of 1–3 weeks) in the time course of the glial scarring, at 6 and 12 weeks post-implantation the tissue response to all of these electrodes was essentially identical. The study concluded

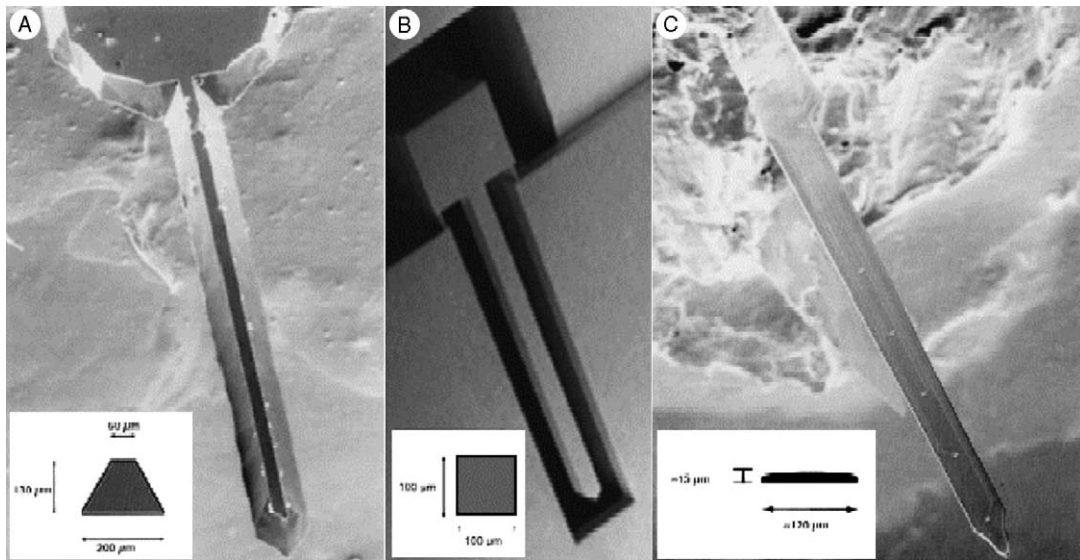


Fig. 9. Different sizes, shapes, and cross-sections of electrodes that produced the same foreign body response and glial scar, suggesting that strategies to improve biocompatibility through purely structural changes in the implant may be ineffective by themselves (from Szarowski et al., 2003).

that while the various geometries may affect the initial wound healing response, glial scar formation was not affected, however, the lack of observable differences may have been due to the low animal number, a lack of controls, and variability of response from animal to animal. Although different materials were not tested by Szarowski et al., other experiments have not shown any significant reduction in the immune response with various metals (Ignatius et al., 1998) or other materials, such as DLC (Singh et al., 2003). Such studies support the shift from a materials science strategy in evading the immune response to strategies focusing on the molecular and cellular biology of the immune response.

4.2. Bioactive molecule strategies

With material science strategies failing to eliminate glial encapsulation, a failure that parallels sensor implants outside the CNS, a number of investigators are examining approaches that manipulate the biological response. Since the proximity of neurons correlates directly with signal strength, strategies that attract, attach, or preserve neurons near recording sites could minimize the effect of the immune response on electrode performance. Such strategies have focused on coating electrodes with bioactive molecules, such as cell adhesion promoting polypeptides or proteins. Neurons use environmental cues to grow, migrate, and stay viable. Some of these cues are in the form of polypeptide motifs on extracellular matrix proteins or on membrane bound molecules of neighboring cells. In addition to intact cell adhesion proteins, such as collagen and fibronectin (Ignatius et al., 1998), several groups have employed cell adhesion peptides, such as RGD (Kam et al., 2002), found in many extracellular proteins, YIGSR (Kam et al., 2002; Cui et al., 2003), and IKVAV (Kam et al., 2002), found in laminin and KHIFS-DDSSE (Kam et al., 2002), found on NCAM (neural-cell adhesion molecule). Studies to establish neuronal reactions to these proteins and peptides have been conducted in vitro. Ignatius et al.

found that poly-D-lysine (a synthetic polypeptide that enhances neural-cell adhesion) and laminin, when co-absorbed on various metals and glass, greatly improved cell attachment, spreading, and growth as compared to uncoated metals. Polyimide, the common insulating material discussed earlier, is also amenable to surface modification with bioactive molecules. Recent studies by Martin have focused on using conducting polymers of polypyrrole and poly(3,4-ethylenedioxythiophene) to provide better contact and a larger surface between the electrode and adjacent neuronal tissue (Cui et al., 2001, 2003; Yang and Martin, 2004). These polymers can be “grown” in a controlled manner through an electrochemical process at the electrode recording sites and can easily incorporate bioactive molecules. Electron micrographs of the sites reveal finger-like fibers of polymer growing out of the gold electrodes, creating a “fuzzy” surface with a large surface area to maximize neuron–electrode interactions. Cui et al. (2001, 2003) incorporated the YIGSR peptide fragment from laminin into the polypyrrole coated recording sites. The peptide-coated sites supported more neuron attachment in vitro compared to peptide-free sites (Cui et al., 2001). When the peptide-incorporated and peptide-free electrodes were chronically implanted in guinea pigs, 83% of peptide-incorporated versus 10% of peptide-free electrode sites showed evidence of neuronal process proximity after 1 week, but the two types of implants produced similar recordings and exhibited similar glial scar reactions (Cui et al., 2003).

Bioactive molecule surface coatings have also been used to either attract or repel glial cells. Neurons in culture will grow on astrocyte monolayers and neuronal processes will extend along tracks provided by astrocytes, oftentimes regardless of the material beneath the astrocytes (Biran et al., 1999, 2003). Attraction of astrocytes and other glial cells could potentially anchor the electrodes in the neural tissue and prevent micromotion, or even block negative components of the glial response (Kam et al., 2002). Kam et al. found that astrocytes adhere preferentially to glass substrates covered with NCAM as compared to

other adhesion molecules. A separate study found that astrocytes also prefer silk-like polymer fragments with fibronectin domains over laminin YIGSR domains, which are preferred by neurons (Cui et al., 2001). Robust growth of glial cells on laminin, collagen, and fibronectin-coated surfaces was also observed (Ignatius et al., 1998). Shain and co-workers have attempted to control and pattern astrocyte adhesion through the deposition of hydrophobic and hydrophilic self assembling monolayers of organosilanes using photolithography and microcontact printing (St. John et al., 1997; Craighead et al., 1998; Kam et al., 1999). Photolithography also allows the manufacture of silicon pillar arrays of varying dimensions (Craighead et al., 1998; Maynard et al., 2000; Turner et al., 2000), which are consistently preferred to smooth silicon by glial cells in vitro. Other studies have tried to prevent astrocyte adhesion in an effort to reduce or eliminate glial scar formation. Singh et al. (2003) found that a dextran coating of DLC-poly-lysine surfaces reduced glial cell adhesion more than 50-fold to $1.41 \pm 1.23\%$ of control surfaces. The study however, was performed using cell lines in vitro, and did not address how such a coating would affect neurons and microglia.

Whether such approaches work in vivo has not been determined. The results indicate a promising direction for research, but it will be necessary to find bioactive molecules that maintain neurons in proximity to the recording surface, minimize astrogliosis, and eliminate chronic microglial activation. To date, IKVAV and YIGSR polypeptide fragments (Cui et al., 2001; Kam et al., 2002) and poly-D-lysine on metal (Ignatius et al., 1998) have elicited different behavior from the various brain cell types, but a “magic bullet” has not been found.

Aside from helping neurons adhere to the electrode recording pads, a more active approach may be to release growth factors or chemoattractants to promote neuronal survival and growth towards the electrode. Numerous chemoattractants and trophic factors act during brain development to facilitate the creation of neural circuits, but growth factor based strategies have generally been disappointing in the adult CNS (Stichel and Muller, 1998a). One promising result was obtained by Kennedy (1989) over 15 years ago when he seeded a standard glass pipette electrode with a piece of sciatic nerve and observed what happened upon chronic implantation of the so-called “cone” electrode. Neural processes from surrounding neurons grew into the electrode tip and this ingrowth could be tracked by the recorded signals. The recorded SNR was often 5–10 times that obtained with wire and silicon electrode arrays and continued for over 12 months, while control electrodes without the sciatic nerve insert showed no neurite ingrowth. Furthermore, the amount of this ingrowth and the resultant single versus multi-unit activity could be controlled by adjusting the size of the cone tip opening. Putatively, the sciatic nerve tissue released growth factors or chemoattractants that caused neurite ingrowth while the electrode design shielded the neurites from the foreign body response and from external electrical noise. Clearly this design cannot be scaled easily to electrode arrays for neural prosthetics, and explanting pieces of PNS tissue for such an array is prohibitive. As a proof of concept however, Kennedy showed the promise of soluble factors in attracting and retaining high quality neural signals.

Standard wound healing suppression and immunosuppression techniques are also options for minimizing the initial immune response and perhaps even the glial scar formation. Both local and systemic administration of corticosteroids and other drugs has been shown to reduce the wound healing response for implants outside of the CNS (Hickey et al., 2002; Yoon et al., 2003). Upon implantation of the UAE into cats by Maynard et al. (2000), two doses of Dexamethasone, a potent suppressant of the wound healing response, were administered 12 h before surgery and again during surgery to control cortical edema, resulting in considerable improvements in implant performance. While promising, the success was likely due to the implantation of the previously mentioned Teflon sheet. Shain et al. (2003) found that peripheral injections of Dexamethasone at the time of electrode insertion greatly attenuated glial scar formation at 1 and 6 weeks as shown by GFAP staining. Some attenuation was seen with local release of Dexamethasone from implanted poly(ethyl-vinyl) acetate strips, but at 6 weeks post-implantation the effect was minor. A peripheral injection of Cyclosporin-A, another potent anti-inflammatory agent, in the same study seemed to increase the glial response.

With the surprising success of Kennedy’s cone electrode came an equally surprising failure. When the same micropipette electrode was filled with a solution of neural growth factor (NGF) in various concentrations to mimic the effect of the sciatic nerve used in the successful trials, no ingrowth was observed (Kennedy, 1989). Instead, a cystic cavity formed around the glass cone electrode, which the author attributed to hyperplastic growth of surrounding tissue that eventually outgrew its blood supply. This failure highlights the importance of developing appropriate drug delivery systems that can capitalize on the positive effects of growth factors or chemoattractants. The normal difficulties of delivering drugs to the CNS are compounded with the difficulties in delivering bioactive molecules over long implantation periods. Current delivery methods include polypyrrole grown on electrode pads and polypeptide-doped polyimide coatings (Cui et al., 2001, 2003). Wells etched into the polyimide electrode developed by Rousche et al. (2001) were filled with dextran as a proof of concept, but could potentially hold other diffusible compounds or hydrogels. Chen et al. (1997) have attempted to address this drug delivery problem with the development of “puffer” probes that incorporate microfluidic channels inside of the electrode shank. These bulk machined silicon probes have multiple 10 μm channels for chemical and drug delivery from orifices situated 2.5 μm from recording sites. There is currently no gating apparatus at the orifices to control of fluid release, but the authors suggest a shutter mechanism is being explored (Chen et al., 1997).

5. Perspectives on the current state of the electrode biocompatibility field

From the large amount of data collected over the past decade on intracortical implant biocompatibility, several trends can be identified for designing future experiments. The complex biological reaction against the implanted electrodes can be separated

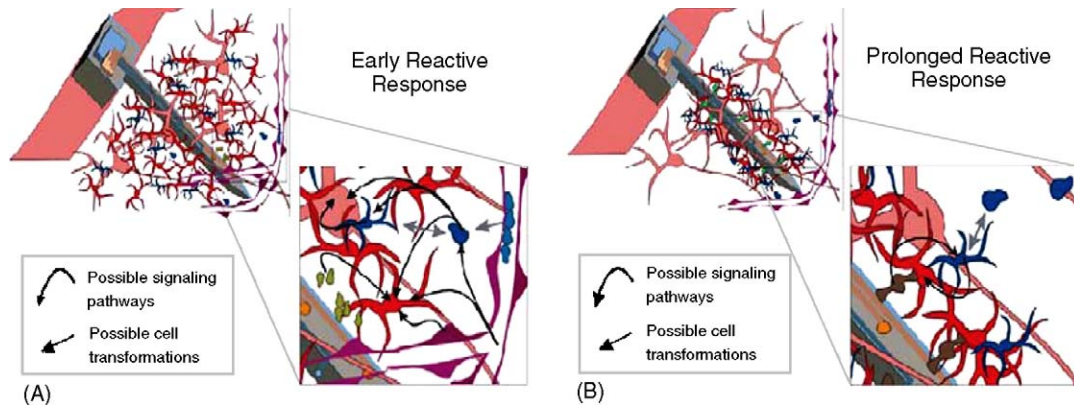


Fig. 10. Possible mechanisms and time course of cellular response to an implant. Neurons are in pink, astrocytes in red, microglia in blue, and vasculature in purple (from Szarowski et al., 2003).

into two immune responses (Fig. 10). The acute phase is a 1–3-week long process in which microglia play a dominant role in response to the insertion trauma. It is unclear how the intensity of the acute response affects subsequent events, which involve both reactive astrocytes and chronically activated microglia. The astrocytic response begins at the time of insertion and is generally completed by 6–8 weeks post-implantation with the development of an encapsulating glial scar. Neuron viability clearly decreases following device insertion, but the question remains whether the neurons that survive the acute reaction and remain in proximity of the chronic foreign body response remain electrically active or viable in the presence of persistent inflammation. The astrocytic scar remodels nearby tissue, thus further separating neurons from the recording electrodes, and possibly increasing electrode impedance. All of these factors most likely contribute to inconsistent performance of recording electrodes and eventually result in a loss of recorded extracellular potentials. The complexity of this response, coupled with a lack of well-controlled experiments and in vitro model systems of reactive gliosis hinders the development of biointeractive strategies. Without a better understanding of the roles that cells, soluble factors, and the extracellular matrix play in both the acute and chronic responses, strategies, such as systemic immunosuppressant application and the incorporation of PNS explants, will remain promising but not clinically applicable. One important step will be developing in vitro cell culture models of reactive gliosis to facilitate a more rational design of therapeutic strategies.

Once a better understanding of the underlying biological processes is obtained, several strategies may be used to combat the immune response. Some researchers believe it is possible to attract neuronal processes to the electrode sites before the astrocytic scar develops, enveloping the neurites within the scar and maintaining a signal. Martin and co-workers are pursuing this with conductive polymer/biomolecule blends grown on the surface of electrode recording pads (Cui et al., 2001, 2003; Cui and Martin, 2003). At the same time neurons are being attracted, suppression of the astrocyte scar formation may prove important. Dextran or other non-adhesive molecules embedded in polyimide coatings have the potential to fulfill this role, but have not been tested in vivo (Singh et al., 2003).

Until recently, attempts to improve electrode reliability in chronic implants have centered on reducing insertion damage and limiting micromotion in vivo (Edell et al., 1992; Yuen and Agnew, 1995; Maynard et al., 2000; Rousche et al., 2001; Shain et al., 2003; Lee et al., 2004). While this approach may have merit, more well-controlled experiments are needed to firmly establish how micromotion contributes to the problem or how it relates to different designs. Alternatively, concentrating efforts to eliminate the chronic reaction rather than trying to minimize the acute reaction may meet with greater success (Reier et al., 1983; Turner et al., 1999; Shain et al., 2003).

The Szarowski et al. (2003) study of the effect of shape, size, and texture on the immune response suggested that a non-biological approach to electrode design may not be sufficient to overcome the biological hurdles of chronic electrode implantation. Consequently, an electrode design that does not couple pharmacological delivery of bioactive molecules or utilize site-specific drug release systems may not overcome the body's immune response. These notions are likely responsible for the shift in next generation electrode array designs from microwire electrodes to silicon-based arrays. However, until these silicon arrays prove successful in vivo the neuroscience field will continue to use the simpler, more constrictive, but more reliable, microwire arrays (Nicollelis et al., 2003).

Finally, it is important to consider the duration over which many studies are conducted. Clinical applications of chronic brain implants must remain active for at least a year if not decades. While decade-long experiments are not feasible in most research environments, studies should be taken out past the 12-week mark to ensure that a full immune response has been mounted against the implant. In vitro studies have always been helpful in guiding the direction of more intensive in vivo experiments, but in the long time periods involved in implant rejection, cell culture experiments are of little use unless they are followed by implantation work. Benchmarking in such studies is also critical to accurately control for experimenter dependent factors. Many biocompatibility studies are conducted with non-functional electrodes rather than with active recording electrodes (Stensaas and Stensaas, 1976; Edell et al., 1992; Yuen and Agnew, 1995; Turner et al., 1999; Szarowski et al., 2003;

Biran et al., 2005) that may more accurately model the brain tissue response. As such, it is difficult to evaluate electrode performance relative to established electrode arrays, and future studies need to benchmark against an established electrically active design to augment the comparison studies already in the literature (Rousche and Normann, 1998; Liu et al., 1999; Maynard et al., 2000; Kipke et al., 2003).

In addition to teaching us about the normal operation of the brain, evidence is accumulating that intracortical multi-channel recording interfaces have significant potential to provide control signals for neuroprosthetic devices ranging from motor control in paralyzed patients to restoring sensory function in the auditory and visual systems (Kennedy and Bakay, 1998; Donoghue, 2002; Serruya et al., 2002; Taylor et al., 2002; Vaughan et al., 2003). The enormous benefits that these implants could deliver have impelled the scientific community to focus on the clinical problems involved in long-term implantation. This focus has paid dividends in the form of more reliable electrode arrays, more accurate measurement techniques, and a better understanding of the processes involved in implant rejection in the CNS. Realizing that they must leverage each others' strengths to create a reliable implant system, neuroscientists specializing in recording neuronal signals and engineers designing implantable electrode systems are increasingly collaborating with each other at various stages of experimentation and design. Such collaboration has yielded a general view that strategies to prevent the foreign body response and glial scar formation are a necessary part of any implant design. While earlier strategies have dealt with minimizing and varying the implant footprint within the CNS, a new focus on bioactive strategies is emerging. This research focus can draw upon the decades of work in the prevention of glial scarring and the initiation of neuronal regeneration in traumatic CNS injuries (Stichel and Muller, 1998b; Fawcett and Asher, 1999; Bovolenta and Feraud-Espinosa, 2000; Hermanns et al., 2001; Schmidt and Leach, 2003; Yick et al., 2003). It is our hope that a rational approach to limiting or eliminating the foreign body response in the CNS through biologically active mediators will augment current implant designs and overcome the obstacles to introducing chronic CNS implants into the clinic.

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